Biochimica et Biophysica Acta 1846 (2014) 121–129

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbacan

Review

Radiation sensitivity of human and murine peripheral blood lymphocytes, stem and progenitor cells

Daniel Heylmann [a], Franz Rödel [b], Thomas Kindler [c], Bernd Kaina [a,*]

[a] Department of Toxicology, University Medical Center, Obere Zahlbacher Straße 67, D-55131 Mainz, Germany
[b] Department of Radiotherapy and Oncology, University Medical Center, Theodor-Stern-Kai 7, D-60590 Frankfurt, Germany
[c] Department of Internal Medicine III, University Medical Center, Langenbeckstraße 1, D-55131 Mainz, Germany

ARTICLE INFO

Article history:
Received 6 February 2014
Received in revised form 2 April 2014
Accepted 26 April 2014
Available online 4 May 2014

Keywords:
Radiation
Cancer therapy
DNA repair
Lymphocytes
Monocytes
Stem cells

ABSTRACT

Immunodeficiency is a severe side effect of radiation therapy, notably at high radiation doses. It may also impact healthy individuals exposed to environmental ionizing radiation. Although it is believed to result from cytotoxicity of bone marrow cells and of immunocompetent cells in the peripheral blood, the response of distinct bone marrow and blood cell subpopulations following exposure to ionizing radiation is not yet fully explored. In this review, we aim to compile the knowledge on radiation sensitivity of immunocompetent cells and to summarize data from bone marrow and peripheral blood cells derived from mouse and human origin. In addition, we address the radiation response of blood stem and progenitor cells. The data indicate that stem cells, T helper cells, cytotoxic T cells, monocytes, neutrophils and, at a high degree, B cells display a radiation sensitive phenotype while regulatory T cells, macrophages, dendritic cells and natural killer cells appear to be more radioresistant. No conclusive data are available for basophil and eosinophil granulocytes. Erythrocytes and thrombocytes, but not their precursors, seem to be highly radioresistant. Overall, the data indicate considerable differences in radiosensitivity of bone marrow and blood normal and malignant cell populations, which are discussed in the light of differential radiation responses resulting in hematotoxicity and related clinical implications.

Contents

1. Introduction

Suppression of hematopoiesis is a severe toxic side effect of radiotherapy (RT) in cancer patients. It also impacts healthy individuals since humans are constantly exposed to external radiation caused by natural background sources, diagnostic procedure or, at the extreme end of the spectrum, nuclear disasters such as the accidents at the Chernobyl and Fukushima nuclear power stations. It is believed that hematotoxicity associated with radiation therapy mainly arises from the impairment of hematopoietic progenitor cells. However, evidence is scarce as limited background information is available regarding the

* Corresponding author.
E-mail address: kaina@uni-mainz.de (B. Kaina).

http://dx.doi.org/10.1016/j.bbcan.2014.04.009
0304-419X/

sensitivity of the different blood normal and malignant cell populations derived from hematopoietic progenitor cells present in the bone marrow. Thus, a major focus of this review is to summarize the current knowledge on radiation responsiveness of different populations of bone marrow and peripheral hematopoietic cells and to discuss reasons for differential radiation responses and possible inferences. As most of the studies arise from human and experimental mouse systems, which display marked differences in response, we will discuss human and mouse data separately.

1.1. Radiation effects on clonogenic survival and self-renewal of murine stem and progenitor cells

Colony formation of irradiated hematopoietic stem and progenitor cells (HSPCs) was taken as an indicator of sensitivity to ionizing radiation (IR); the data are compiled in Table 1 (for phenotypic classification stem and precursor cells see Fig. 1S). Imai and Nakao [1] were among the first to report that after a 1.5 to 3 Gy total body irradiation (TBI), bone marrow multipotent progenitor cells i.e. the mixed colony-forming units (CFU-Mix ~ CFU-GEMM) with radiobiological characteristics of $D_0 = 1.44$ Gy (D_0 = dose reducing to 37% of cell survival) and progenitors for granulocytes and monocytes ($D_0 = 1.57$ Gy) are relatively resistant to IR while pluripotent and self-renewal hematopoietic stem cells CFU-S-10 (in the spleen of mice at 10 days after transplantation) are radiosensitive ($D_0 = 0.81$ Gy). Erythroid burst forming units (BFU)-E ($D_0 = 0.68$ Gy) and erythroid progenitors CFU-E ($D_0 = 0.53$ Gy) displayed the most radiation sensitive phenotype. This is in line with a significant decrease of erythrocytes in peripheral blood following IR, although erythrocytes themselves are relatively radiation resistant. The progenitors for fibroblasts (CFU-F) was the most resistant population ($D_0 = 2.57$ Gy). CFU-Mix, BFU-E and CFU-S-10 cells were identified as slow recovering cells, which do not reach physiological levels even at 28 days after IR, while CFU-E recovered quickly. A third group of progenitors, comprising CFU-C and CFU-F, decreased more slowly and recovered to normal levels on day 10 to 14 [1].

Later on it was reported that the most primitive totipotent stem cells with bone marrow-repopulating ability (MRA), that have self-renewal competence, showed the highest level of radiation resistance compared to their differentiated descendants [2,3]. Thus, totipotent MRA_CFU-S-12 (day-twelve) splenic CFU, which showed a high differentiation potential thus forming colonies after re-transplantation in bone marrow and spleen, have nearly the same D_0 value (1.18 Gy) as ex vivo-growing primitive MRA_CFU-Cultures (1.13 Gy). CFU-S-12, which display early multipotent stem cells and more matured MRAs, were more radiosensitive ($D_0 = 0.95$ Gy) and the less primitive stem cell colonies CFU-S-7 was the most sensitive ($D_0 = 0.71$ Gy) population. Moreover, in vitro studies assigned myeloid progenitors CFU-Culture (mixed populations of CFU-GM, CFU-G and CFU-M) a low level of radiosensitivity ($D_0 = 1.47$ Gy) [3] (see Fig. 2S). These data confirm the early observations of Imai and Nakao [1]. Collectively, the results indicate that early and primitive self-renewal totipotent hematopoietic stem cells are more radiation resistant than multipotent stem cells. By contrast, opposite results were published in another investigation [4,5]. The more mature stem cells CFU-S-8 showed higher radiation resistance compared to primitive pluripotent stem cells CFU-S-12. This study, however, was performed with a dose <1 Gy in fetal murine spleens [4,

Table 1
Radiation response of murine and human hematopoietic stem cells, progenitor cells and peripheral blood cells. Explanation of symbols: h, hours; d, days; w, weeks; and y, years.

Radiosensitivity	Species	Dose	Time	Ref.
Lin-CD117+Sca1+ ≥ Lin-CD117+Sca1-	Mouse	4 Gy	18 h	[6]
BFU-E > CFU-E > CFU-S10 > CFU-Mix > CFU-GM Recover ability: CFU-E > CFU-GM > CFU-Mix, BFU-E, CFU-S10	Mouse	1.5–3 Gy	4 w	[1]
CFU-S7 > CFU-S12 > MRA > CFU-Culture	Mouse	0.5–5 Gy	7–12 d	[3]
CD34+CD38- > CD34+CD38+	Human	5 Gy	16 h	[26]
Lin-CD34+CD38-CD90+/− > CD34+CD38+	Human	3 Gy	–	[25]
CFU-Mix > CFU-GM > BFU-E CFU-Meg (spring) > CFU-Meg (autumn) CFU-GM (autumn) > CFU-GM (spring) CFU-Mix (peripheral blood) > CFU-Mix (cord blood)	Human	0.5–2 Gy 2 Gy	–	[27,28,30]
Immature CFU-Meg > mature CFU-Meg	Human	–	–	[31]
B cells > T cells > NK CTL > Th	Mouse	3 Gy	–	[15]
B cells ≫ DCs, NK, Treg Th > CTL, Treg	Mouse	2 Gy >0.1 Gy	–	[16,18]
Th > Treg	Mouse	2 Gy	4 h–3 d	[17]
T cells, monocytes, granulocytes > thrombocytes > erythrocytes	Mouse	8 Gy	4 h 12 d	[23]
Monocytes ≫ DCs > macrophages	Human	0.5–2 Gy	24 h	[37]
B cells > memory Th > naive CTL > naive Th > NK Th > CTL	Human	2 Gy	24 h	[40]
B cells > CTL > Th Th (male) > Th (female)	Human	0.5–2 Gy	18 h	[42]
B cells > NK cells Th and CTL no difference	Human cancer patients	50 Gy (2 Gy fractions)	5 w	[41]
Leucocytes > platelets > erythrocytes (hemoglobin)	Human cancer patients	39–70 Gy (1.8–2 Gy fractions)	1–7 w	[34]
B cells ≫ T cells Recover ability: B cells ≫ T cells	Human cancer patients	–	–	[38]
Lymphocytes > neutrophils, monocytes > platelets > erythrocytes (hemoglobin)	Human cancer patients	35 Gy (1.75 Gy fractions) 31 Gy (1.25 fractions)	28 d 41 d	[33]
Incidence: Leucopenia > neutropenia, anemia > thrombocytopenia	Human cancer patients	40–50 Gy (1.8 Gy fractions) + cisplatin	–	[39]
Decrease in T cells;Th most sensitive; increase in B cells and immature T cells; on granulocytes and NK cells no effects	Human Hiroshima Nagasaki	>1 Gy	20 y	[44]
Decrease in T cells CTL depletion only in low-dose exposed Recovery ability: Treg > Th/CTL Granulocytes, megakaryocytes > erythrocytes	Human Chernobyl	<9 Gy	1–5 y	[44]

5] while the study alluded to above was performed with spleens of adult mice (Fig. 2S). This may indicate that differences do exist in radiation sensitivity between hematopoietic murine fetal stem cells and stem cells from mature mice.

Other studies revealed that both irradiation dose and endpoint of investigation have an impact on radiation sensitivity. Thus, with a dose of 4 Gy, 18 h post-exposure and the endpoint apoptosis, no clear differences in the induction of cell death was evident between hematopoietic stem lineage cluster of differentiation 117+, stem cell antigen-1+ (Lin-CD117+Sca-1+) cells (44.8%) and their progenitors (Lin-CD117+Sca-1-) (38.4%) [6] (see also Fig. 1S). It is of interest to note that the induction of senescence in hematopoietic stem cells was observed several weeks after IR, which was characterized by p53, $p21^{Cip1/Waf1}$, $p16^{Ink4}$, and beta-galactosidase (SA-β-gal) expression [6,7]. Thus, it is tempting to speculate that senescence plays a protective role as it allows more time for the repair of IR induced DNA damage for cells in order to recover from treatment.

Proceeding long-term effects of IR on HSPCs have also been investigated. Irradiation of mice (7 Gy or 4 × 4.5 Gy) resulted in a decrease of the progenitors CFU-GM, BFU-E and the multipotent progenitor CFU-S in the bone marrow [8,9]. This decline (30% of non-treated level) persisted for one year. A slight recovery to 60% of control level was detected for CFU-GM and BFU-E 20 months after exposure. BFU-E progenitors in spleen showed the most pronounced decrease in the first month after IR when compared with CFU-S and CFU-GM, demonstrating acute radiosensitivity [8]. The recovery of progenitor cells occurred faster in younger mice (8 days old) than in older mice. In younger mice, the number of CFU-S and BFU-E cells returned to normal levels one year after radiation exposure. It seems that mice of low age are more effective in compensating the loss of blood cells after IR than older mice [8].

Moreover, mice displayed elevated values of γ-H2AX foci as biomarker for DNA double-strand breaks (DSB) in bone marrow stem cells one year after a 7 Gy exposure, which was not observed in stem cells of mock-irradiated mice [10]. Furthermore, 24 h after irradiation with 1 Gy, stem cells of older mice (16 month) showed increased numbers of persisting γ-H2AX foci than irradiated stem cells of younger mice. Thus, one may assume that stem cells of younger mice possess a more effective ability in repairing DSBs than older one. There was no difference in stem cell DSB repair capacity observed between untreated and 7 Gy pre-treated older mice [10].

An accumulation of DNA damage presumably due to a defect in the DNA damage response was also observed in the early stem cell compartment Lin-Sca-1+cKit+ (cKit = CD117) [11,12]. IR-induced chronic oxidative stress is considered to be one of the sources for residual damage observed in mice [13]. Irradiation also causes cycling and an increase in stem cell divisions probably for maintaining hematopoiesis [14].

1.2. Radiation effects on murine peripheral blood cell counts

An overview of radiation sensitivity of murine peripheral blood cells is depicted in Table 1. Kajioka *et al.* [15] described acute effects of TBI on the immune system of mice exposed to a single dose of 3 Gy. At four days after exposure they observed the highest degree of immunosuppression and demonstrated that CD19+ B cells were the most sensitive, CD3+ T cells were moderately sensitive and natural killer cells (NK) the most resistant cell population. Similarly, B cells were reported to be the most sensitive population, while dendritic cells (DCs), NKs and regulatory T cells (Treg) displayed a more resistant phenotype. Within the T cell population, CD4+ T helper (Th) cells were more sensitive than CD8+ cytotoxic lymphocytes (CTL) and Treg [16]. By contrast, CTL were reported to comprise the most radiosensitive population in the T cell compartment in another study [15]. This may indicate that differences in radiosensitivity especially between Th and CTL vary depending on study and experimental setup. Consistently, however, CD4+CD25+ Treg were one of the most radioresistant lymphocyte subpopulations after TBI of mice [15,17,18].

The radiation resistance of Treg might implicate a negative consequence in radiotherapy. Thus, it is conceivable that ionizing radiation causes a shift to tumor-protective Treg by depleting the radiosensitive T cells (CTL and Th), which are tumor defending. Indeed, this has been shown to be the case [17]. In line with this, elimination of Treg by anti-CD25 antibody treatment improved radiation-induced tumor regression [18]. Radioresistance of Treg might also impact the inflammatory response. Thus, using a murine model of collagen-induced arthritis, it was reported that repeated 0.5 Gy of IR attenuates the pathology of the disease. This was accompanied by a significant increase of Treg and suppression in the production of cytokines (IL-6, IL-17) by Th [19]. Furthermore, there is a report showing that after low-dose irradiation (LDR) differences in sensitivities were observed with CTL to be more radiosensitive than Treg [20]. Moreover, LDR (0.01–0.1 Gy) reduces spontaneous apoptosis in NKs and DCs and increased T cell activation [16]. Despite elevated radiosensitivity, CTL became activated in mice following fractionated low-dose exposure (0.2 Gy), which was not observed for Th [21], an effect that still requires mechanistic explanation. Recent data showed that apart from inducing cell death by apoptosis in peripheral blood cells, LDR has been shown to modulate adhesion molecules and inflammatory nuclear factor kappa B (NF-κB) expression in endothelial cells, which impacts the adhesion of leucocytes to the endothel (most pronounced after 0.5 Gy) thus contributing to an anti-inflammatory clinical effect [22].

Regarding high-dose irradiation, Garg *et al.* [23] reported on decreased numbers in circulating T lymphocytes, monocytes and granulocytes 4 h after treatment with 8 Gy TBI. One month later, monocytes reached pre-treatment levels and granulocytes recovered even above the physiological level. The thrombocyte count declined between day 7 and 21 and erythrocytes were reduced at day 14 but recovered by day 21. A dose of 7 Gy TBI resulted in a significant decrease in erythrocyte count one year after exposure in mice [10]. Thus, IR seems to damage radiosensitive precursors of erythrocytes (CFU-E/BFU-E) but not erythrocytes directly, resulting in decreased erythrocyte levels several months later.

Finally, the group reported on severe changes in the intestinal immune system [23]. Four hours after IR, a decrease of macrophages and B cell counts was observed in the intestine. Macrophage levels recovered quickly within one week, whereas B cells recovery was delayed, requiring 21 to 30 days to return to basal levels. CD4+ T cells decreased more slowly reaching a nadir after 3.5 days and started recovering after 14 days. Neutrophil granulocyte levels were stable at 24 h after exposure, but showed a severe and transient decrease from day 3.5 to day 21. Interestingly, neutrophils showed a very quick recovery above basal levels at day 30. Given the fact that neutrophils have a short half-life in peripheral blood, these data may indicate that the immediate precursors of neutrophils are relatively radiation sensitive while the distal precursors are radiation resistant.

1.3. Radiation effects on clonogenic survival and self-renewal of human stem and progenitor cells

Human HSPCs are usually considered CD34+ cells, which are present in bone marrow, cord blood and, at low amounts, in the peripheral blood (2.3×10^6 per litre) [24]. The human stem cell population, however, is more complex (Fig. 1S). It is divided into primitive human hematopoietic stem cells (HSC) Lin-CD34+CD38-CD90+CD45RA-, multipotent progenitors (MPP) Lin-CD34+CD38-CD90-CD45RA- and oligopotent progenitors CD34+ CD38+. The radiation sensitivity of these three populations has been assessed (Table 1). HSC and MPP underwent significantly more apoptosis and showed reduced clonogenic survival compared to their oligopotent progenitors following IR [25,26]. Moreover, delayed DSB rejoining and a higher number of residual γH2AX foci as well as an increased expression of p53, apoptosis-

stimulating protein of p53 (ASPP1) and enhanced level of apoptosis were observed in the HSC and MPP population compared to oligopotent progenitors when exposed to IR. HSC were protected against IR-induced cell death by inactivation of p53 or overexpression of the anti-apoptotic protein Bcl-2 [25]. Further, it can be speculated that impaired DSB repair by delayed non-homologous end joining (NHEJ) and a higher level of residual DNA damage gives rise to a higher frequency of p53 triggered apoptosis in HSC and MPP, but not in CD34+CD38+ progenitor cells. Although the biological relevance of the different sensitivities of the CD34+ stem cell populations remains elusive, these data reveal that even within the so-called "stem cell compartment" significant differences do exist in the sensitivity to IR (Fig. 1).

Regarding CFUs generated from human mononuclear cells from peripheral blood, a ranking in sensitivity was reported following a 0.5 and 2 Gy exposure: CFU-Mix > CFU-GM > BFU-E [27–29]. It should be mentioned here that CFU-Mix differentiated from cord blood are more radioresistant than CFU-Mix from peripheral blood, showing radiation responses similar to BFU-E [28,30]. Furthermore, a significantly increased radiation sensitivity of total HSPCs was observed in male but not in female blood donors, when isolated stem cells were irradiated with a dose of 0.5 Gy. It was also not observed in blood samples obtained from males and females irradiated with 2 Gy [28,30]. Additionally, seasonal variations were evident: CFU-GM differentiated from cord blood were significantly more resistant in newborns with birth at spring compared to newborns in autumn and winter, while the opposite was true for CFU-Meg. These differences may be due to variable cytokine levels induced by seasonal aeroallergens. Also, gender specific variations were obvious: CFU-GM in males were more radioresistant than CFU-GM in females, and CFU-Meg were more radioresistant in females than in males [28,30]. With respect to megakaryocyte precursors, immature CFU-Meg (D_0 = 0.56–0.77 Gy) are more radiosensitive than mature CFU-Meg (D_0 = 0.86–1.12 Gy) [31]. This was basically confirmed in follow-up studies with Chernobyl victims, showing that megakaryoblasts and megakaryocytes are radioresistant [32].

1.4. Radiation effects on human peripheral blood cell counts

Most data arise from peripheral blood cells obtained from cancer patient following radiation therapy. Plowman [33] studied patients with Hodgkin's disease treated with mantle and inverted Y field RT (total dose of 35 Gy in 28 fractions, Fig. 2A). Immediately following mantle field irradiation, lymphocytes dropped to ~20%, while neutrophils dropped to ~50% of pre-therapy counts (PTC). Monocytes decreased to ~70% of PTC. Although not addressed in this study, a decline in lymphocyte counts may be a consequence of radiation-induced cell death by apoptosis or, alternatively, it is related to an

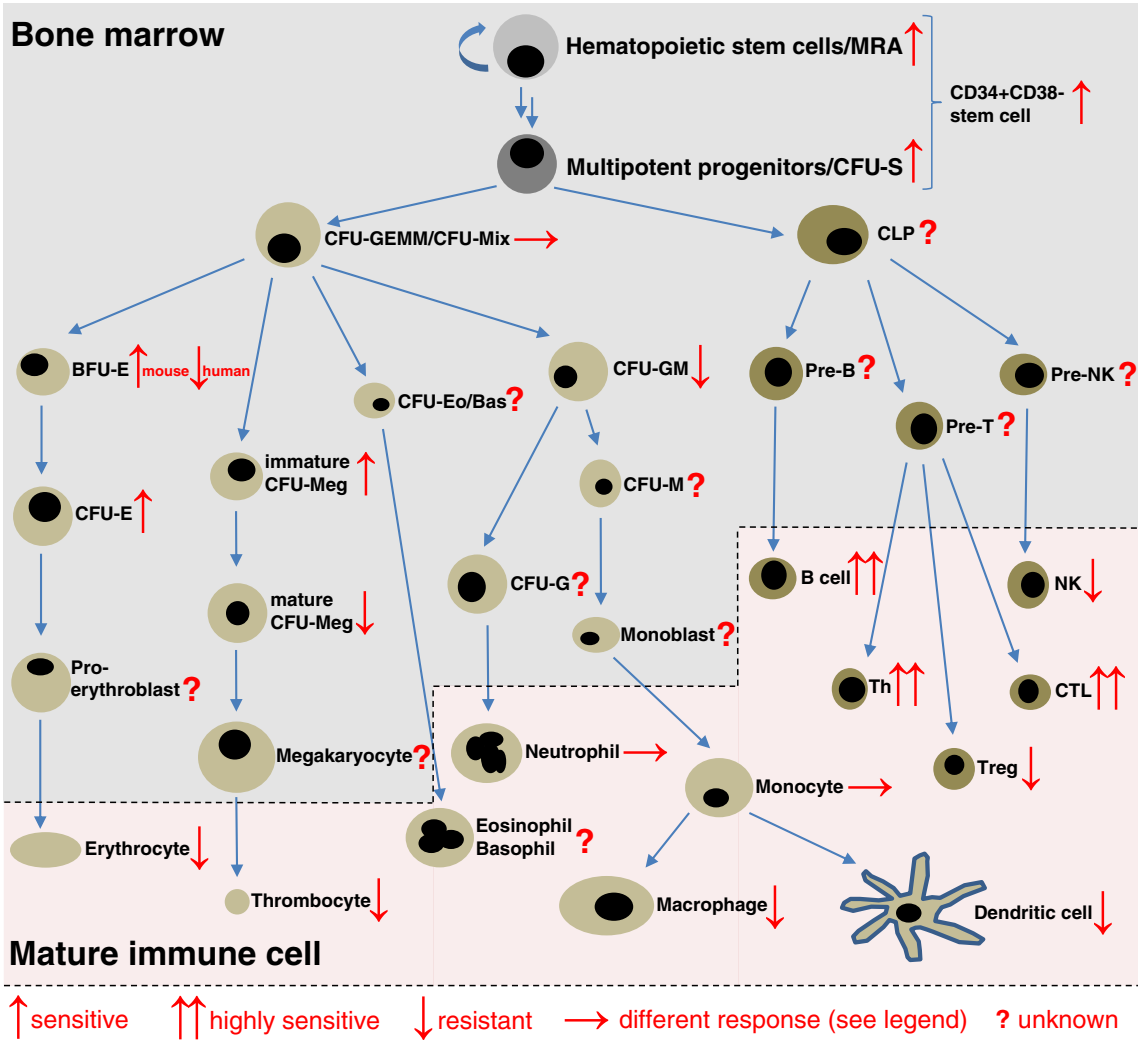

Fig. 1. Radiosensitivity of hematopoietic blood cell populations in the bone marrow and periphery (data from Table 1). Red arrows indicate the response: ↑, high radiosensitivity; ↓, low radiosensitivity. CFU-GEMM (CFU-Mix) (median radiosensitivity, →) are less radiosensitive than HSCs, but more radiosensitive than CFU-GM. The progenitors described as CFU-GEMM, CFU-Mix and CFU-S are not always clearly separated from each other. Monocytes (→) and neutrophils (→) are more radioresistant than lymphocytes, but more radiosensitive as erythrocytes and thrombocytes.

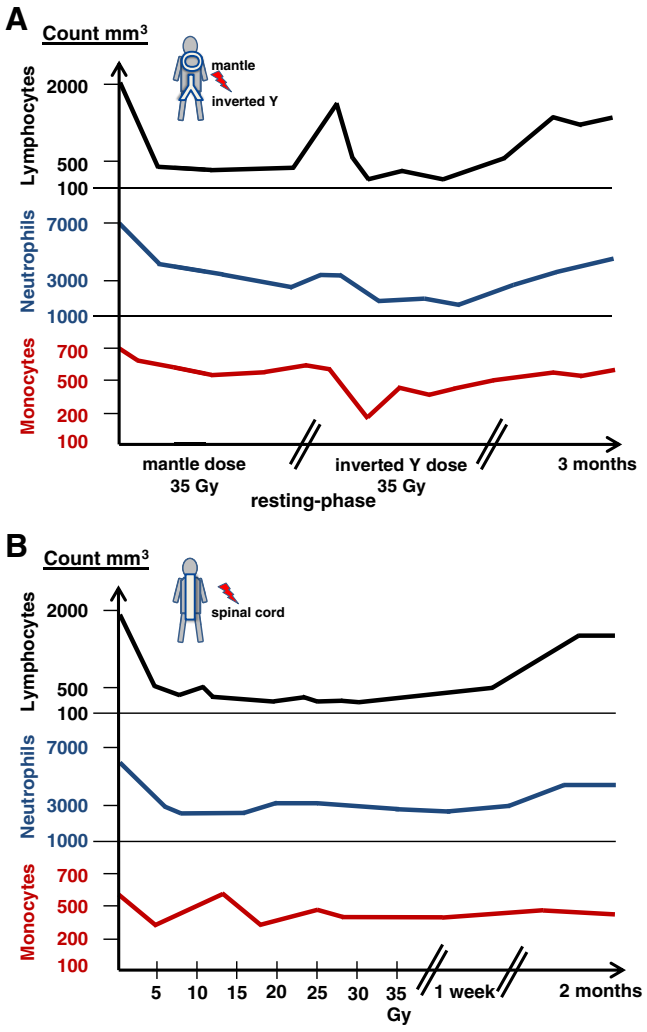

Fig. 2. Cell counts of lymphocytes, neutrophils and monocytes in patients during radiotherapy (data schematically adapted from Plowman [33]). A) Hodgkin's disease patients received RT of total 35 Gy to mantle area and 35 Gy to inverted Y area. Lymphocytes show the strongest decrease in cell count directly after start of mantle irradiation maintaining low levels afterwards. Neutrophils dropped to ~50% pre-therapy count (PTC). Monocyte counts are less affected and increased after a weak decline in the mid of mantle therapy. During the resting-phase (1–2 months) lymphocytes recover completely to PTC, neutrophils incompletely to ~50% PTC. Immediately after the onset of inverted Y irradiation, lymphocytes, neutrophils and monocytes showed a severe decrease. Monocytes recovered quickly to ~50% pre-therapy level and increased transiently to pre-therapy level up to 3 months after therapy. Lymphocytes and neutrophils recovered at the end of inverted Y irradiation, but the recovery was faster in lymphocytes. B) Medulloblastoma patients received a total of 35 Gy to the spinal cord. A massive decrease was visible after a cumulative dose of ~5 Gy for lymphocytes, neutrophils and monocytes. Lymphocytes maintained a very low level up to 1 week after therapy, where they start to recover continuously to below PTC. Neutrophil counts recovered slightly after cumulative ~15 Gy to ~50% PTC and begun to recover 1 week after therapy with an endpoint below PTC. Monocytes showed wave-like recovery and decreasing steps, at ~12.5 Gy even above PTC. They reached a level below PTC at the end of therapy, maintaining this plateau for 2 months.

increased extravasation and tissue infiltration of leucocytes from peripheral blood or their impaired mobilization from bone marrow precursors.

In the resting-phase (1–2 months) between mantle and inverted Y field RT, lymphocytes and monocytes recovered to ~80% of PTC whereas neutrophils remained at a low (~50%) level. Based on these recovery abilities, it can be inferred that precursors of neutrophils (CFU-G) are more sensitive to IR than precursors of monocyte (CFU-M) and lymphoid progenitors (CLP). Immediately following inverted Y RT, lymphocytes decreased to a nadir of ~15% PTC while neutrophils halved their resting-phase level. Monocytes were much more affected in this radiation field dropping to ~30% PTC with a fast recovery afterwards. Lymphocytes and neutrophils started recovering at the end of therapy and reached PTC levels almost 3 month after RT. Recovery of lymphocytes occurred faster than that of neutrophils. As mentioned above, it can be assumed that progenitors of lymphocytes (CLP) are more radioresistant than the immediate neutrophil progenitors CFU-G [33].

The response of patients with medulloblastoma receiving a total dose of 35 Gy within 35 days to the spinal cord was also reported [33]. The decline of lymphocytes, neutrophils and monocytes is summed up in Fig. 2B. The data indicate that depletion of monocytes is quickly compensated during therapy while neutrophils recover one week after therapy. Lymphocytes recovered to PTC reaching a plateau one month after the onset of therapy. Overall, the following sequence of radiation sensitivity can be inferred from published data: lymphocytes > neutrophils ~ monocytes > platelets > erythrocytes. A similar ranking in radiosensitivity was confirmed in a subsequent study [34] (Table 1). Tubiana et al. [35] reported that in patients treated with TBI, granulocytes were the most sensitive blood cell population showing a rapid decline within 8 days following exposure. In another study, a significant increase in circulating monocytes and eosinophils was reported in patients received RT for malignant disease of the pelvis and thorax with a cumulative dose of 35 Gy [36], indicating that compensatory mechanisms for monocyte and eosinophil depletion work quiet efficiently. Although only limited data are available at present, it appears that the blood monocyte population declines immediately after RT, but recovers afterwards, which is in line with the study reviewed above [33]. Notably, human monocytes are more radiosensitive than macrophages and DCs derived from them [37], indicating that peripheral monocytes are radiation sensitive and become radioresistant during a 6–8 day differentiation period. To the best of our knowledge, there are no data available regarding the radiosensitivity of human monocytes in the bone marrow and their progenitors.

A severe and acute lymphocytopenia after irradiation of iliac and paraaortic lymph nodes was noted by Heier *et al.* [38]. They also reported that B lymphocytes are more sensitive than T lymphocytes. Blood lymphocytes reached physiologic levels 5 to 10 years after RT with B cells recovering faster than T cells, supporting the speculation that B cell precursors (Pre-B) are less vulnerable to IR than T cell precursors (Pre-T) [38].

Finally, Mell *et al.* [39] described the effect of intensity-modulated pelvic RT for cervical cancer (39.6–50.4 Gy total dose) and concurrent cisplatin chemotherapy (40 mg/m^2 per week) on acute hematologic toxicity. The authors deduced a sequence of grade 2 or worse anemic incidence following this schedule as follows: leukopenia > neutropenia > anemia > thrombocytopenia. Overall, the data reported above might indicate that thrombocytes recover faster than leukocytes and neutrophils, which may indicate that the progenitors of thrombocytes, i.e. the megakaryoblasts and megakaryocytes, are radiation resistant. It should be recalled that thrombocytes lack a nucleus and thus DNA damage dependent survival pathways will not be activated. Although immediate hematotoxicity is often attributed to a direct effect of radiation to the peripheral blood, it remains to be clarified whether cytotoxicity of progenitor cells in the bone marrow contributes to the observed decline in blood cell numbers.

Flow cytometry is an accurate method for assessing cell death frequencies of individual blood cell populations by the use of specific surface markers. By determining the frequency of apoptosis in different lymphocyte subpopulations of peripheral blood mononuclear cells (PBMCs) irradiated *in vitro* (24 h, 2 Gy), the following order of radiosensitivity was observed: B cells (CD20+) > memory T cells (CD4+CD45RA-) > naive CTL (CD8+CD45RA+) > naive Th (CD4+CD45RA+) > NK (CD3-CD56+). Furthermore, Th with a CD3+CD4+ phenotype were more sensitive than CD3+CD8+ CTL [40], which is contradictory to data obtained in mice (Table 1). These data were confirmed following *in vivo* irradiation indicating that B cells being the most and NK cells being the

least sensitive fraction in cancer patients who received RT to the pelvis. However, they did not report significant differences between Th and CTL counts during RT [41]. In contrast, Schmitz et al. [42] showed that CD8+ CTL were more sensitive than CD4+ Th cells. They also demonstrated that radiosensitivity of CTL increased with age and that Th cells obtained from woman and irradiated *ex vivo* were less radiosensitive than those derived from man. These findings suggest that gender, age and possibly other factors (e.g. nutrition) might explain the different results with regard to radiosensitivity of CTL and Th lymphocytes.

Another question to be addressed is whether the ratio of Th/CTL under co-cultivation conditions has an impact on radiation response. Different Th/CTL ratios had no influence on CTL sensitivity, but Th cells showed lower numbers of apoptotic cells with increasing ratios. This effect is considered to originate from different cytokine levels varying with the amount of Th and CTL cells in mixed cultures [43]. These results further suggest that analysis of radiation sensitivity of blood cell populations should also take into consideration the relative amounts of different cell populations. It is reasonable to expand the screening for radiosensitivity to all the peripheral blood populations. Thus, a direct comparison of the different cell types with respect to radiation sensitivity and signaling pathways will be achieved.

1.5. Nuclear accidents

Substantial information regarding radiation effects on the immune system was obtained by analysis of atomic bomb survivors in Hiroshima and Nagasaki. Akiyama [44] reviewed the effects of irradiation on the immune system focusing on late effects, i.e. 20 years after the insult. Long-term abnormalities observed in survivors exposed to high doses (>1 Gy) pertain to T and B cell populations. In general, the number of Th cells was reduced in the peripheral blood (especially the CD4+ CD45RA+ population) and functional defects were observed. Interestingly, no effects in cell number were evident in CD8+ T cell subsets (CTL). On the contrary, immature T cell subsets (CD3+CD8-CD4-) and B cells increased significantly while NK cells and granulocytes did not show any radiation effects compared to healthy controls [45–51]. With respect to acute effects, evidence exists for a rapid decline in the number of lymphocytes, antibody and complement production shortly after exposure [52] and a decrease in the number of neutrophils and monocytes with a nadir of 30 days [53]. This results in severe hematopoietic dysfunction concomitant with a reduction in phagocytosis and deficiency in antigen recognition by T cells, antibody production and impaired activation of neutrophils and monocytes [54–60].

The accident at the Chernobyl nuclear power plant in 1986 also provided data regarding IR-induced blood cell toxicity. Akiyama [44] reported a decline in T cells in individuals with acute radiation disease [61–63]. Cleanup personnel exposed to low-dose irradiation (0.37 Gy) showed a normal or even slightly increased number of T cells in peripheral blood, which normalized within 1–2 years. Granulocytes and megakaryocytes exhibited an earlier recovery than erythrocytes, which were slow to recover and were retarded during the first year in patients with radiation injuries of the skin [63]. The recovery of Th cells occurred later than that of Treg cells (suppressor T cells). As with atomic bomb survivors, a drop of CTL was detectable only in those individuals exposed to low doses while a decrease of Th cell number was observed in individuals exposed to high (up to 9 Gy) doses [64]. The elevated radiosensitivity of CTL and Th cells may be used as a biomarker of exposure if linked with γ-H2AX staining. Thus, γ-H2AX formation in human peripheral blood lymphocytes was reported as a bio-dosimetric determinant with a linear relationship between the initial dose and foci per nucleus after exposure to IR [65].

1.6. DNA damage response in human blood cells

In a recent study, the sensitivity of primary human monocytes derived from peripheral blood of healthy donors was compared with the sensitivity of macrophages and dendritic cells derived from them by cytokine maturation. In these investigations monocytes were shown to be hypersensitive to IR, displaying a high rate of apoptosis even after low-dose exposure <2 Gy. This was attributed to impaired base excision (BER) and DSB repair. Monocytes displayed high levels of single-strand breaks (SSB) and DSB following IR in contrast to macrophages and DCs. The DNA damage response and cell death pathways were strongly activated in monocytes after exposure to reactive oxygen species (ROS) as analyzed by phosphorylation of ATM, ATR, CHK1, CHK2 and p53 and activation of caspases 8, 3 and 7 [37]. Furthermore, monocytes lack the expression of the BER factors X-ray repair cross-complementing protein (XRCC1), poly (ADP-ribose) polymerase 1 (PARP-1) and ligase IIIα and, therefore, were not able to perform the last steps of BER and DSB repair by B (backup) NHEJ. Monocytes also lack DNA protein kinase catalytic subunit (DNA-PKcs), which is involved in NHEJ, making them defective for this repair pathway. DSB and BER repair capacity was elevated once monocytes were stimulated to differentiate into DCs and macrophages by treatment with the cytokines IL-4 and GM-CSF. These data indicate that human monocytes, but not macrophages and dendritic cells, are vulnerable to IR. This further supports the view that DNA repair capacity may differ in normal and malignant hematopoietic cell populations to result different radiation sensitivities.

The inhibition of DNA-PKcs activity by phosphatase inhibitors (e.g. microcystin-LR) was further shown to negatively impact on the repair of radiation-induced DSB in lymphocytes, which was reflected by a reduced formation of γ-H2AX foci, an increased level of chromosomal aberrations and increased radiosensitivity [66–68]. Although T and B cells differ in radiation sensitivity, they display equal levels in basal DNA-PKcs activity and DSB rejoining [69]. Of note, B cells seem to be defective in nucleotide excision repair (NER), which is presumably due to impaired DNA binding protein DDB2 and ubiquitin-like modifier activating enzyme 1 (Ube1) activity [70]. With a closer look at granulocytes, expression arrays for DNA repair genes demonstrated that eosinophils, but not neutrophils, have the ability to repair DSB and SSB [71]. This fits well to the data mentioned above showing that neutrophils exhibit similar radiation sensitivity as monocytes. Furthermore, nucleophosmin (NPM1), which regulates the p53 response to DNA damage, was upregulated in eosinophils, but not in neutrophils. Therefore NPM1 is considered a resistance marker for eosinophils [72].

1.7. Radiation response of leukemic cell populations

The effect of radiation exposure on different malignant human cells was extensively reviewed [73]. In summary, a Burkitt's lymphoma cell line derived from a lymphatic B cell tumor displayed an enhanced radiation response ($D_0 = 1.38$ Gy) compared to normal human lymphocytes ($D_0 = 1.95$ Gy) and B lymphoblastoid cells ($D_0 = 2.46$ Gy). Several groups demonstrated a dominant role of the p53 functional status in the context of radiosensitivity [74]. For example, a p53 wildtype (wt) HSB-2 (Pre-T) cell line was more radiosensitive than p53 wt/mt MOLT-4 (Pre-T) cells, p53 mutant (mt) CEM (T) and p53 deleted HL-60 (pre-myelocyte) cells. Likewise, TK6 (derived from a B cell lymphoma) and HSB-2 cells, both p53 wt, are more sensitive than the p53 mutated derivative WTK1 [75]. Thus, mutations in TP53 may confer an impaired radiation response and, as a consequence, a radioresistant phenotype, which likely results from a reduced apoptosis capacity. However, impaired p53 function in TK6 cells following transfection with human papillomavirus E6 gene revealed only little effect on the radiation response [76]. In contrast, mantle cell lymphomas (MCL, derived from naive B cells) comprise highly radiation sensitive tumor cells despite nonfunctional p53. Different MCL cell lines that lack p53 function displayed imbalanced NHEJ (variations in Ku70 and DNA-PKcs protein expressions) and die by p53-independent apoptosis. Additionally, mutations and deletions in ATM alleles were observed, suggesting that

Table 2
Radiation response of leukemic cell populations. Explanation of abbreviations: wt, wildtype; mt, mutant; ALL, acute lymphoid leukemia; CLL, chronic lymphoid leukemia; T, T cell lineage; B, B cell lineage;AML, acute myeloid leukemia.

Radiosensitivity	Species	Dose	Time	Ref.
Burkitt's lymphoma > normal lymphocytes > B lymphoblastoid	Human	–	–	[73]
HSB-2 (p53 wt, Pre-T), TK6 (p53 wt, B) > MOLT-4 (p53 wt, mt, Pre-T) > Reh (early B), CEM (p53 mt, T) > HL-60 (p53 deleted, Pre-myelocyte)	Human	1–8 Gy	–	[74,75]
ALL T > MOLT-4/3/4c (ALL T) > CCRF-HSB-2 (ALL T) > thymocytes ≫ ALL null > RPMI8392 (B), RPMI 1788 (B), CCRF-SB (ALL, B), B411-4 (B), CLL B (B) > PBMCs > stimulated lymphocytes > CLL T	Human	[^{51}Cr] labeling	4–8 h	[78]
176 (AML) > HL50 (pro-myelocytic leukemia) > 45 (ALL) > K562 (erythroleukemia)	Human	–	–	[80]
Jurkat (acute T) > K562 (chronic myelogenous leukemia) > PBMCs	Human	0.8–5 Gy	3–72 h	[81]

ATM might be an upstream key factor for an alternative mechanism of apoptosis independent of p53 [77].

Data listed in Table 2 indicate that leukemic blood cells of different origin display a clear difference in radiation sensitivity (see also Ref. [78–81]). Notably, acute lymphoblastic/myeloid leukemic cells and T-cell leukemia cells (immature T cell derivatives) are sensitive towards IR while cells from chronic lymphocytic leukemia such as B cells and chronic myeloid leukemia cells were reported to be more radioresistant. Again, the p53 status and apoptosis signaling pathways appear to play a major role in determining radiation sensitivity, as killing effects in follicular lymphoma, one of the most radiation sensitive malignancy, was related to p53 activation, survivin down regulation and caspase-8/-9 activation [82,83]. For B-cell chronic lymphocytic leukemia (B-CLL) radiation resistant and sensitive populations have been reported, which differ in NHEJ and DNA-PKcs activity with an increased level in the resistant cells. Inhibition of DNA-PK$_{CS}$ by NU 7026 or, more generally by inhibition of phosphatidylinositide 3-kinases (PIK-3) by Wortmannin, evoked a radiation sensitive phenotype of B-CLL cells [84], indicating DSB repair utilizing the NHEJ pathway involved in B-CLL radioresistance.

2. Conclusions

The compiled data depicted in Fig. 1 (and Tables 1 and 2) display the differential sensitivity of human normal and malignant hematopoietic cell populations. Stem cells, Th cells, CTL, monocytes, neutrophil granulocytes and especially B cells display a radiation sensitive phenotype, while Treg, macrophages, DCs, NK cells and thrombocytes (particularly their immediate precursor, the megakaryocytes) are more radioresistant. No conclusive data are available at present for basophil and eosinophil granulocytes. Erythrocytes seem to be radioresistant, but not their progenitors (CFU-E and BFU-E) in the bone marrow. Caution is urged if data obtained in mice are extrapolated to humans. Thus, the progenitors of erythrocytes in mice (CFU-E and BFU-E) seem to be radiation sensitive while in human they are more resistant. As to progenitor cells, totipotent stem cells and multipotent progenitors CD34+CD38- turn out to be more radiosensitive than mature oligopotent progenitors CD34+CD38+ cells. Of note, the most primitive totipotent stem cells seem to be more radioresistant than the mature multipotent stem cell compartment. There might also be inter-individual differences that have to be taken into account. Some results provide evidence that the more primitive and multipotent progenitors are more radiosensitive than mature CFU-GM. Therefore, it is conceivable that CFU-GM became radioresistant during their differentiation from CFU-GEMM.

To the best of our knowledge, it is not known whether the immediate progenitors of granulocytes (neutrophils) and monocytes, CFU-G and CFU-M, are radioresistant or sensitive. This is also true for the precursors of lymphocytes including Pre-T, Pre-B and CLP (common lymphoid progenitors) for which reliable data are not yet available. They are required, however, for explaining the differential responses of T and B cells, which possess the highest radiosensitivity of mature blood cells. There appears to be a ranking in radiosensitivity such as Pre-T > Pre-B and that CFU-G (neutrophil precursor) > CFU-M ~ CFU-Eo, which needs to be substantiated in future experiments.

In the context of malignancies, a differential radiation response of immune cells may have significant impact on the anticancer immune response. Thus, Treg are considered to inhibit the anti-cancer activity of the immune system mediated by effector T cells, eosinophils and NK cells. The radiation response of cells is a result of a plethora of functions, including reactive oxygen species (ROS) and reactive nitrogen species (NOS) scavenging, DNA damage induction and repair, signaling evoked by DNA damage and the activation of apoptosis, necrosis, necroptosis, saturated autophagy and other cell death pathways. Since a present paradigm in radiation biology describes DNA targeted effects and DNA repair at the heart of defense against genotoxin-induced cell death, it would be of utmost importance to elucidate the DNA repair capacity in the different leukocyte populations. This knowledge is required for optimizing passive and active immunotherapy. Also, multimodal therapies with chemical genotoxicants and molecular targeted strategies might be optimized in anti-cancer treatment.

Acknowledgement

We gratefully acknowledge Heinz Schmidberger for helpful comments and Adam Thomas for the critical reading. Work was supported by a grant of the DFG to BK (DFG KA 724/20-2).

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbcan.2014.04.009.

References

[1] Y. Imai, I. Nakao, In vivo radiosensitivity and recovery pattern of the hematopoietic precursor cells and stem cells in mouse bone marrow, Exp. Hematol. 15 (1987) 890–895.
[2] E.I. Meijne, R.J. van der Winden-van Groenewegen, R.E. Ploemacher, O. Vos, J.A. David, R. Huiskamp, The effects of x-irradiation on hematopoietic stem cell compartments in the mouse, Exp. Hematol. 19 (1991) 617–623.
[3] E.I.M. Meijne, Radiosensitivity of the murine hemopoietic stem cell compartment, The effects of 1 MeV fission neutrons and 300 kV X-rays, Reprografische Dienst Energy Research Foundation, Petten, 1996.
[4] P. Uma Devi, Radiosensitivity of the developing haemopoietic system in mammals and its adult consequences: animal studies, Br. J. Radiol. 76 (2003) 366–372.
[5] P. Uma Devi, M. Hossain, K.S. Bisht, Effect of gamma radiation on the foetal haemopoietic system in the mouse, Int. J. Radiat. Biol. 74 (1998) 639–646.
[6] A. Meng, Y. Wang, G. Van Zant, D. Zhou, Ionizing radiation and busulfan induce premature senescence in murine bone marrow hematopoietic cells, Cancer Res. 63 (2003) 5414–5419.
[7] Y. Wang, B.A. Schulte, A.C. LaRue, M. Ogawa, D. Zhou, Total body irradiation selectively induces murine hematopoietic stem cell senescence, Blood 107 (2006) 358–366.
[8] T. Grande, S. Gaitan, C. Tejero, J.A. Bueren, Residual haematopoietic damage in adult and 8 day-old mice exposed to 7 Gy of X-rays, Int. J. Radiat. Biol. 63 (1993) 59–67.
[9] J.H. Hendry, C.X. Xu, N.G. Testa, A cellular analysis of residual hemopoietic deficiencies in mice after 4 repeated doses of 4.5 Gray X rays, Int. J. Radiat. Oncol. Biol. Phys. 9 (1983) 1641–1646.
[10] J. Vavrova, Z. Sinkorova, M. Rezacova, A. Tichy, S. Filip, J. Mokry, E. Lukasova, Irradiated stem cells and ageing of the haematopoietic system, Radiat. Environ. Biophys. 51 (2012) 205–213.

[11] D.J. Rossi, D. Bryder, J. Seita, A. Nussenzweig, J. Hoeijmakers, I.L. Weissman, Deficiencies in DNA damage repair limit the function of haematopoietic stem cells with age, Nature 447 (2007) 725–729.
[12] A.J. Simonnet, J. Nehme, P. Vaigot, V. Barroca, P. Leboulch, D. Tronik-Le Roux, Phenotypic and functional changes induced in hematopoietic stem/progenitor cells after gamma-ray radiation exposure, Stem Cells 27 (2009) 1400–1409.
[13] Y. Wang, L. Liu, S.K. Pazhanisamy, H. Li, A. Meng, D. Zhou, Total body irradiation causes residual bone marrow injury by induction of persistent oxidative stress in murine hematopoietic stem cells, Free Radic. Biol. Med. 48 (2010) 348–356.
[14] N. Ban, M. Kai, Implication of replicative stress-related stem cell ageing in radiation-induced murine leukaemia, Br. J. Cancer 101 (2009) 363–371.
[15] E.H. Kajioka, M.L. Andres, J. Li, X.W. Mao, M.F. Moyers, G.A. Nelson, J.M. Slater, D.S. Gridley, Acute effects of whole-body proton irradiation on the immune system of the mouse, Radiat. Res. 153 (2000) 587–594.
[16] E.N. Bogdandi, A. Balogh, N. Felgyinszki, T. Szatmari, E. Persa, G. Hildebrandt, G. Safrany, K. Lumniczky, Effects of low-dose radiation on the immune system of mice after total-body irradiation, Radiat. Res. 174 (2010) 480–489.
[17] A. Balogh, E. Persa, E.N. Bogdandi, A. Benedek, H. Hegyesi, G. Safrany, K. Lumniczky, The effect of ionizing radiation on the homeostasis and functional integrity of murine splenic regulatory T cells, Inflamm. Res. 62 (2013) 201–212.
[18] E.L. Kachikwu, K.S. Iwamoto, Y.P. Liao, J.J. DeMarco, N. Agazaryan, J.S. Economou, W. H. McBride, D. Schaue, Radiation enhances regulatory T cell representation, Int. J. Radiat. Oncol. Biol. Phys. 81 (2011) 1128–1135.
[19] H. Nakatsukasa, M. Tsukimoto, A. Tokunaga, S. Kojima, Repeated gamma irradiation attenuates collagen-induced arthritis via up-regulation of regulatory T cells but not by damaging lymphocytes directly, Radiat. Res. 174 (2010) 313–324.
[20] K. Lumniczky, E.N. Bogdandi, T. Szatmari, G. Safrany, Effects of low dose radiation on the main immune parameters in healthy mice, Radioprotection 43 (2008) 156.
[21] R. Pandey, B.S. Shankar, D. Sharma, K.B. Sainis, Low dose radiation induced immunomodulation: effect on macrophages and CD8+ T cells, Int. J. Radiat. Biol. 81 (2005) 801–812.
[22] F. Rodel, B. Frey, U. Gaipl, L. Keilholz, C. Fournier, K. Manda, H. Schollnberger, G. Hildebrandt, C. Rodel, Modulation of inflammatory immune reactions by low-dose ionizing radiation: molecular mechanisms and clinical application, Curr. Med. Chem. 19 (2012) 1741–1750.
[23] S. Garg, M. Boerma, J. Wang, Q. Fu, D.S. Loose, K.S. Kumar, M. Hauer-Jensen, Influence of sublethal total-body irradiation on immune cell populations in the intestinal mucosa, Radiat. Res. 173 (2010) 469–478.
[24] F. Passamonti, L. Vanelli, L. Malabarba, E. Rumi, E. Pungolino, L. Malcovati, C. Pascutto, E. Morra, M. Lazzarino, M. Cazzola, Clinical utility of the absolute number of circulating CD34-positive cells in patients with chronic myeloproliferative disorders, Haematologica 88 (2003) 1123–1129.
[25] M. Milyavsky, O.I. Gan, M. Trottier, M. Komosa, O. Tabach, F. Notta, E. Lechman, K.G. Hermans, K. Eppert, Z. Konovalova, O. Ornatsky, E. Domany, M.S. Meyn, J.E. Dick, A distinctive DNA damage response in human hematopoietic stem cells reveals an apoptosis-independent role for p53 in self-renewal, Cell Stem Cell 7 (2010) 186–197.
[26] T. Hayashi, I. Hayashi, T. Shinohara, Y. Morishita, H. Nagamura, Y. Kusunoki, S. Kyoizumi, T. Seyama, K. Nakachi, Radiation-induced apoptosis of stem/progenitor cells in human umbilical cord blood is associated with alterations in reactive oxygen and intracellular pH, Mutat. Res. 556 (2004) 83–91.
[27] K. Kato, M. Kuwabara, I. Kashiwakura, The influence of gender- and age-related differences in the radiosensitivity of hematopoietic progenitor cells detected in steady-state human peripheral blood, J. Radiat. Res. 52 (2011) 293–299.
[28] K. Kato, A. Omori, I. Kashiwakura, Radiosensitivity of human haematopoietic stem/progenitor cells, J. Radiol. Prot. 33 (2013) 71–80.
[29] A. Oriya, K. Takahashi, O. Inanami, T. Miura, Y. Abe, M. Kuwabara, I. Kashiwakura, Individual differences in the radiosensitivity of hematopoietic progenitor cells detected in steady-state human peripheral blood, J. Radiat. Res. 49 (2008) 113–121.
[30] A. Omori, T. Chiba, I. Kashiwakura, Relationship between radiosensitivity of human neonatal hematopoietic stem/progenitor cells and individual maternal/neonatal obstetric factors, J. Radiat. Res. 51 (2010) 755–763.
[31] I. Kashiwakura, M. Kuwabara, O. Inanami, M. Murakami, Y. Hayase, T.A. Takahashi, Y. Takagi, Radiation sensitivity of megakaryocyte colony-forming cells in human placental and umbilical cord blood, Radiat. Res. 153 (2000) 144–152.
[32] G.P. Gruzdev, A.S. Chistopol'skii, L.A. Suvorova, Radiosensitivity and postradiation kinetics of megakaryocyte release of the bone marrow (analysis based on data of the Chernobyl AES accident sequelae), Radiats. Biol. Radioecol. 36 (1996) 250–263.
[33] P.N. Plowman, The effects of conventionally fractionated, extended portal radiotherapy on the human peripheral blood count, Int. J. Radiat. Oncol. Biol. Phys. 9 (1983) 829–839.
[34] F.E. Yang, F. Vaida, L. Ignacio, A. Houghton, J. Nauityal, H. Halpern, H. Sutton, S. Vijayakumar, Analysis of weekly complete blood counts in patients receiving standard fractionated partial body radiation therapy, Int. J. Radiat. Oncol. Biol. Phys. 33 (1995) 607–617.
[35] M. Tubiana, E. Frindel, H. Croizat, C. Parmentier, Effects of radiations on bone marrow, Pathol. Biol. (Paris) 27 (1979) 326–334.
[36] M. Rotman, H. Ansley, L. Rogow, S. Stowe, Monocytosis: a new observation during radiotherapy, Int. J. Radiat. Oncol. Biol. Phys. 2 (1977) 117–121.
[37] M. Bauer, M. Goldstein, M. Christmann, H. Becker, D. Heylmann, B. Kaina, Human monocytes are severely impaired in base and DNA double-strand break repair that renders them vulnerable to oxidative stress, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 21105–21110.
[38] H.E. Heier, I. Christensen, S.S. Froland, A. Engeset, Early and late effects of irradiation for seminoma testis on the number of blood lymphocytes and their B and T subpopulations, Lymphology 8 (1975) 69–74.
[39] L.K. Mell, J.D. Kochanski, J.C. Roeske, J.J. Haslam, N. Mehta, S.D. Yamada, J.A. Hurteau, Y.C. Collins, E. Lengyel, A.J. Mundt, Dosimetric predictors of acute hematologic toxicity in cervical cancer patients treated with concurrent cisplatin and intensity-modulated pelvic radiotherapy, Int. J. Radiat. Oncol. Biol. Phys. 66 (2006) 1356–1365.
[40] J. Philippe, H. Louagie, H. Thierens, A. Vral, M. Cornelissen, L. De Ridder, Quantification of apoptosis in lymphocyte subsets and effect of apoptosis on apparent expression of membrane antigens, Cytometry 29 (1997) 242–249.
[41] H. Louagie, M. Van Eijkeren, J. Philippe, H. Thierens, L. de Ridder, Changes in peripheral blood lymphocyte subsets in patients undergoing radiotherapy, Int. J. Radiat. Biol. 75 (1999) 767–771.
[42] A. Schmitz, J. Bayer, N. Dechamps, G. Thomas, Intrinsic susceptibility to radiation-induced apoptosis of human lymphocyte subpopulations, Int. J. Radiat. Oncol. Biol. Phys. 57 (2003) 769–778.
[43] R.C. Wilkins, B.C. Kutzner, M. Truong, J.R. McLean, The effect of the ratio of CD4+ to CD8+ T-cells on radiation-induced apoptosis in human lymphocyte subpopulations, Int. J. Radiat. Biol. 78 (2002) 681–688.
[44] M. Akiyama, Late effects of radiation on the human immune system: an overview of immune response among the atomic-bomb survivors, Int. J. Radiat. Biol. 68 (1995) 497–508.
[45] Y. Kusunoki, S. Kyoizumi, Y. Fukuda, H. Huang, M. Saito, K. Ozaki, Y. Hirai, M. Akiyama, Immune responses to Epstein–Barr virus in atomic bomb survivors: study of precursor frequency of cytotoxic lymphocytes and titer levels of anti-Epstein–Barr virus-related antibodies, Radiat. Res. 138 (1994) 127–132.
[46] Y. Kusunoki, M. Akiyama, S. Kyoizumi, E.T. Bloom, T. Makinodan, Age-related alteration in the composition of immunocompetent blood cells in atomic bomb survivors, Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med. 53 (1988) 189–198.
[47] S. Sasagawa, Y. Yoshimoto, E. Toyota, S. Neriishi, M. Yamakido, M. Matsuo, Y. Hosoda, S.C. Finch, Phagocytic and bactericidal activities of leukocytes in whole blood from atomic bomb survivors, Radiat. Res. 124 (1990) 103–106.
[48] S.C. Finch, A review of immunologic and infectious disease studies at ABCC-RERF, RERF, Technical Report, 1979, pp. 22–79.
[49] R.F. Barreras, S.C. Finch, Peripheral blood leucocyte phagocytosis and respiratory response to certain macromolecular substances in the ABCC JNIH Adult Health Study, Hiroshima, ABCC Technical Report, 1974.
[50] R.K. Blaisdell, K. Akamoto, Review of ABCC hematologic studies, 1947–59, ABCC Technical Report, 1966, pp. 25–66.
[51] J.W. Hollingsworth, H.B. Hamilton, Blood bactericidal activity, Hiroshima, ABCC Technical Report, 1960, pp. 14–60.
[52] K. Kimura, N. Azuma, M. Goto, S. Hotta, K. Notake, Bacteriological Study of Serum in A-bomb Patients, Medical Report on Atomic Bomb Effects. Part 2, Science Council of Japan, Japan Society for the Promotion of Science, Tokyo, 1953. 806–810.
[53] T. Ohkita, Acute effects, J. Radiat. Res. (Tokyo) 16 (1975) 49–66.
[54] T. Kikuchi, S. Kimoto, Clinical Phase of A-bomb Sickness, Medical Report on Atomic Bomb Effects. Part 2, Science Council of Japan, Japan Society for the Promotion of Science, Tokyo, 1953. 1580–1642.
[55] A.W. Oughtersen, S. Warren, Hematology of atomic bomb injuries, pathology of atomic bomb injuries, Medical Effects of the Atomic Bomb in Japan, National Nuclear Energy Series Division, VIII, McGraw-Hill, 1956. 191–284.
[56] K. Sassa, Y. Nakao, G. Kobayashi, S. Kato, Y. Yano, M. Komiya, Hematological study of A-bomb radiation sickness, Medical Report on Atomic Bomb Effects. Part 1, Science Council of Japan, Japan Society for the Promotion of Science, Tokyo, 1953, pp. 649–662.
[57] F.M. Snell, J.V. Neel, K. Ishibashi, Hematologic studies in Hiroshima and a control city two years after the atomic bombing, ABCC Technical Report, 1959, pp. 27–59.
[58] KureNavalStation, Investigation concerning A-bomb in Hiroshima city (medical aspects), Medical Report on Atomic Bomb Effects. Part 1, Science Council of Japan, Japan Society for the Promotion of Science, Tokyo, 1953, pp. 423–436.
[59] C. Tamagawa, Autopsy records on 19 cases of A-bomb sickness in Hiroshima city, Medical Report on Atomic Bomb Effects. Part 2, Science Council of Japan, Japan Society for the Promotion of Science, Tokyo, 1953, pp. 1497–1561.
[60] K. Wakisaki, Clinical study of A-bomb patients, Japanese Textbook of Hematology. Part 3, Japanese Society of Hematology, Maruzen, Tokyo, 1964. 600–629.
[61] V.G. Bebeshko, A.A. Chumak, D.A. Bazika, Immunocompetent cells in patients who recovered from acute radiation sickness and children evacuated from the 30-km zone, Proceedings of the National Conference on Scientific-Practical Aspects of Health Promotion of People Exposed to Radiation, Ministry of Health BSSR, Minsk, 1991, pp. 119–120.
[62] I.E. Gurmanchuk, G.D. Kharitonik, E.E. Sigalovich, Frequency and spectrum of immune deficient and immunopathological conditions in children living in radionuclide contaminated areas, Proceedings of the National Conference on Scientific-Practical Aspects of Health Promotion of People Exposed to Radiation, Ministry of Health BSSR, Minsk, 1991, pp. 124–125.
[63] G.P. Gruzdyev, A.S. Chistopolsky, L. Suvorova, On the question of radiosensitivity of cellular elements of erythropoietic components of bone marrow, Radiobiol. Radioecol. 34 (1993) 587.
[64] A.A. Yarilin, I.M. Belyakov, O.I. Kusmenok, V.Y. Arshinov, A.V. Simonova, N.M. Nadezhina, E.V. Gnezditskaya, Late T cell deficiency in victims of the Chernobyl radiation accident: possible mechanisms of induction, Int. J. Radiat. Biol. 63 (1993) 519–528.
[65] C.E. Redon, J.S. Dickey, W.M. Bonner, O.A. Sedelnikova, Gamma-H2AX as a biomarker of DNA damage induced by ionizing radiation in human peripheral blood lymphocytes and artificial skin, Adv. Space Res. 43 (2009) 1171–1178.
[66] P. Douglas, G.P. Sapkota, N. Morrice, Y. Yu, A.A. Goodarzi, D. Merkle, K. Meek, D.R. Alessi, S.P. Lees-Miller, Identification of in vitro and in vivo phosphorylation sites in the catalytic subunit of the DNA-dependent protein kinase, Biochem. J. 368 (2002) 243–251.

[67] A. Lankoff, J. Bialczyk, D. Dziga, W.W. Carmichael, I. Gradzka, H. Lisowska, T. Kuszewski, S. Gozdz, I. Piorun, A. Wojcik, The repair of gamma-radiation-induced DNA damage is inhibited by microcystin-LR, the PP1 and PP2A phosphatase inhibitor, Mutagenesis 21 (2006) 83–90.
[68] T. Wechsler, B.P. Chen, R. Harper, K. Morotomi-Yano, B.C. Huang, K. Meek, J.E. Cleaver, D.J. Chen, M. Wabl, DNA-PKcs function regulated specifically by protein phosphatase 5, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 1247–1252.
[69] A. Vral, H. Thierens, P. Bryant, L. De Ridder, A higher micronucleus yield in B-versus T-cells after low-dose gamma-irradiation is not linked with defective Ku86 protein, Int. J. Radiat. Biol. 77 (2001) 329–339.
[70] N. Hyka-Nouspikel, K. Lemonidis, W.T. Lu, T. Nouspikel, Circulating human B lymphocytes are deficient in nucleotide excision repair and accumulate mutations upon proliferation, Blood 117 (2011) 6277–6286.
[71] S. Salati, E. Bianchi, R. Zini, E. Tenedini, D. Quaglino, R. Manfredini, S. Ferrari, Eosinophils, but not neutrophils, exhibit an efficient DNA repair machinery and high nucleolar activity, Haematologica 92 (2007) 1311–1318.
[72] J. Li, X. Zhang, D.P. Sejas, G.C. Bagby, Q. Pang, Hypoxia-induced nucleophosmin protects cell death through inhibition of p53, J. Biol. Chem. 279 (2004) 41275–41279.
[73] P.J. Deschavanne, B. Fertil, A review of human cell radiosensitivity in vitro, Int. J. Radiat. Oncol. Biol. Phys. 34 (1996) 251–266.
[74] D.R. Aldridge, I.R. Radford, Explaining differences in sensitivity to killing by ionizing radiation between human lymphoid cell lines, Cancer Res. 58 (1998) 2817–2824.
[75] P.L. Olive, J.P. Banath, R.E. Durand, Development of apoptosis and polyploidy in human lymphoblast cells as a function of position in the cell cycle at the time of irradiation, Radiat. Res. 146 (1996) 595–602.
[76] Y. Yu, C.Y. Li, J.B. Little, Abrogation of p53 function by HPV16 E6 gene delays apoptosis and enhances mutagenesis but does not alter radiosensitivity in TK6 human lymphoblast cells, Oncogene 14 (1997) 1661–1667.
[77] R. M'Kacher, A. Bennaceur, F. Farace, A. Lauge, L.F. Plassa, E. Wittmer, J. Dossou, D. Violot, E. Deutsch, J. Bourhis, D. Stoppa-Lyonnet, V. Ribrag, P. Carde, C. Parmentier, A. Bernheim, A.G. Turhan, Multiple molecular mechanisms contribute to radiation sensitivity in mantle cell lymphoma, Oncogene 22 (2003) 7905–7912.
[78] S. Nakazawa, J. Minowada, T. Tsubota, L.F. Sinks, Profound radiosensitivity in "leukemic" T-cell lines and T-cell-type acute lymphoblastic leukemia demonstrated by sodium [51Cr]chromate labeling, Cancer Res. 38 (1978) 1661–1666.
[79] A. Nevoie, M. Pascariu, D. Jitaru, I. Ivanov, D. Constantinescu, E. Carasevici, T. Luchian, Investigation of apoptosis in normal and leukemic cells induced by X-ray irradiation, Dig. J. Nanomater. Biostruct. 6 (2011) 259–264.
[80] R.R. Weichselbaum, J.S. Greenberger, A. Schmidt, A. Karpas, W.C. Moloney, J.B. Little, In vitro radiosensitivity of human leukemia cell lines, Radiology 139 (1981) 485–487.
[81] T.E. Wheldon, The radiobiological basis of total body irradiation, Br. J. Radiol. 70 (1997) 1204–1207.
[82] R.L. Haas, Low dose radiotherapy in indolent lymphomas, enough is enough, Hematol. Oncol. 27 (2009) 71–81.
[83] L. Knoops, R. Haas, S. de Kemp, D. Majoor, A. Broeks, E. Eldering, J.P. de Boer, M. Verheij, C. van Ostrom, A. de Vries, L. van't Veer, D. de Jong, In vivo p53 response and immune reaction underlie highly effective low-dose radiotherapy in follicular lymphoma, Blood 110 (2007) 1116–1122.
[84] L. Deriano, O. Guipaud, H. Merle-Beral, J.L. Binet, M. Ricoul, G. Potocki-Veronese, V. Favaudon, Z. Maciorowski, C. Muller, B. Salles, L. Sabatier, J. Delic, Human chronic lymphocytic leukemia B cells can escape DNA damage-induced apoptosis through the nonhomologous end-joining DNA repair pathway, Blood 105 (2005) 4776–4783